Reports

Intraocular Pressure in Inbred Mouse Strains

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Purpose. To develop a protocol to measure the intraocular pressure (IOP) of living mice and to determine the IOP of genetically different mouse strains.

Methods. Eyes of anesthetized animals were cannulated with a very fine fluid-filled glass microneedle. The microneedle was connected to a pressure transducer, and the pressure signal was analyzed with a computer system. Intraocular pressures of male C3H/HeJ, C57BL/6J, A/J, and BALB/cJ mice were determined.

Results. Differences in IOP were detected between genetically distinct mouse strains maintained in virtually identical environments. C3H/HeJ was the strain with the highest average IOP (13.7 ± 0.8 mm Hg). This strain average was 1.4 mm Hg higher than that for C57BL/6J (12.3 ± 0.5 mm Hg; $P = 0.14$), 4.3 mm Hg higher than that for A/J (9.4 ± 0.5 mm Hg; $P < 0.001$), and 6 mm Hg higher than that for BALB/cJ (7.7 ± 0.5 mm Hg; $P < 0.001$).

Conclusions. The authors have developed an accurate and reliable procedure for measuring intraocular pressure in living mice. This procedure can detect IOP differences between groups of mice that differ by genotype. Invest Ophthalmol Vis Sci. 1997;38:249–253.

Glaucoma is a group of complex diseases involving the death of retinal ganglion cells and the degeneration of the optic nerve head. Glaucoma is the leading cause of blindness in black Americans and the second leading cause of blindness in the United States.[1] It is often associated with high intraocular pressure (IOP) that results from an increased resistance to drainage of the aqueous humor. Idiopathic or primary open-angle glaucoma usually exhibits multifactorial inheritance, and many studies indicate that relatives of patients with primary open-angle glaucoma have a higher risk for the disease than the general population.[1] Two of the best-known risk factors for glaucoma, IOP and aqueous humor outflow, follow multifactorial inheritance patterns.[2]

Glaucoma traditionally is viewed as a disease in which deleteriously high IOP results in optic nerve damage over time.[1] Intraocular pressure, however, is not the only factor. Nerve damage does not develop in most persons who have high IOP for extended periods of time, but in others, nerve damage develops despite IOP levels in the normal range.[1] The factors determining susceptibility of the retina and optic nerve to glaucomatous damage are unknown. Some studies implicate diabetes, vascular hypertension, arterial hypotension, cardiovascular disease, sex, and race in glaucomatous neuropathy. There are, however, studies that find no association between the above factors and glaucoma, and it is unclear which factors actually interact with IOP to cause damage.[3] The genetic and pathophysiological basis of glaucoma is poorly understood.

The ability to alter the mouse genome by adding transgenes or altering endogenous genes makes mice the most advanced mammalian system for assessing the function of identified genes and for assessing the consequences of mutating or overexpressing these genes.[4,5] The hundreds of inbred mouse strains, the high density of genetic markers mapped on mouse chromosomes, the ability to breed mice in the desired fashion, and the ability to control environments make the analysis of complex multifactorial traits more practical in mice than in any other mammal.[4] We have found no previous reports of IOP measurement in mice. The development of an accurate and reliable method to measure IOP in mice should facilitate greatly the identification of genes involved in IOP regulation and the susceptibility of the retina and the optic nerve to glaucomatous damage. We describe here a procedure that allows the measurement of IOP in mice.

MATERIALS AND METHODS. Animal Husbandry and Experimental Design. All experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male mice of strains C3H/HeJ (C3H), C57BL/6J (B6), A/J, and BALB/cJ (BALB) were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice of various genetic backgrounds were used in the experiments to validate the measurement procedure and to test the effect of microneedle insertion on IOP. Mice were received 3 weeks after birth and were housed in adjacent cages covered loosely with lexon filters and containing white pine bedding. The environment was kept at 21°C with a 14-hour light–

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10-hour dark cycle. All mice were fed NIH31 (4% fat) chow ad libitum. Animals ranged between 9 and 11 weeks of age at the time of IOP measurement. To prevent differences caused by the time of measurement, mice of different strains were intermixed during all measurement periods.

Standardization of the Plane of Anesthesia. The mice were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (9 mg/kg). Anesthesia was administered to one mouse at a time. Each mouse was monitored carefully to assess the state of anesthesia. After the mouse lost consciousness and failed to respond to touch, IOP was measured as soon as possible (typically within 2 to 3 minutes of loss of consciousness). Then anesthesia was injected and IOP was measured in the next mouse. This protocol ensured IOP assessment of animals that were in a similar plane of anesthesia and in a similar physiologic state. In addition, it helped to control for interindividual variability in the rate of metabolism and susceptibility to the anesthetic agents and to control for variability in absorption rates after intraperitoneal administration.

Instrumentation. A diagrammatic representation of the system is shown in Figure 1. A water-filled microneedle was used to cannulate the eyes. To make the microneedle, borosilicate glass (OD 1.0 mm, ID 0.58 mm; Sutter Instruments, Novato, CA) was pulled with a pipette puller (Flaming Brown [Sutter Instruments]), and the tip was cut at an outer diameter of 50 μm using a microforge (DeFonbrune, General Scientific, Redhill Surrey, England). The tip was then beveled to 45° with a microgrinder (Narishige, Tokyo, Japan) and sharpened on a microforge. The microneedle was connected to a DTX pressure transducer (Viggo Spectramed, Oxnard, CA). The pressure transducer relayed its signal to a PM1000 amplifier (CWE, Inc, Ardmore, PA) that was connected to a DI200 data acquisition card (Dataq Instruments) and a computer. Windaq data acquisition and playback software (Dataq Instruments, Akron, OH) were used to collect and analyze the data. Before measurement, the system was calibrated by use of a mercury manometer.

Validation. To validate this system over a range of pressures, one investigator randomly varied the IOP up and down between 1 and 45 mm Hg by use of a water-filled micrometer syringe connected to the eye and to a water-filled manometer (Fig. 1B). This investigator could not see the computer reading generated by a pressure transducer, which was connected to the same eye independent of the water manometer. A second investigator, who could not see the manometer reading, recorded the transducer-generated pressure. One hundred twenty readings were obtained in six mice (20 per eye). To determine whether IOP is altered on entering the eye, a duplicate measurement system was constructed. Intraocular pressure measurement was performed as usual, except that the microneedle was left in the anterior chamber after a pressure reading was obtained. The microneedle of the second system was inserted into the anterior chamber. The pressure recorded by the second microneedle was compared to the reading obtained before its insertion.

Intraocular Pressure Measurement. As soon as the mice were anesthetized sufficiently, they were placed on a surgical platform and were immobilized with stainless steel head and leg holders. When manipulating the animals, we were careful to avoid pressure on the neck that could alter IOP. A drop of phosphate-buffered saline (PBS) was placed on each eye to prevent corneal dehydration and to alter refraction and allow a clear view into the anterior chamber. The left eye was viewed under a dissecting microscope, and the microneedle tip was placed inside its drop of PBS. At this point, the pressure reading was zeroed. The tip of the microneedle was inserted into the anterior

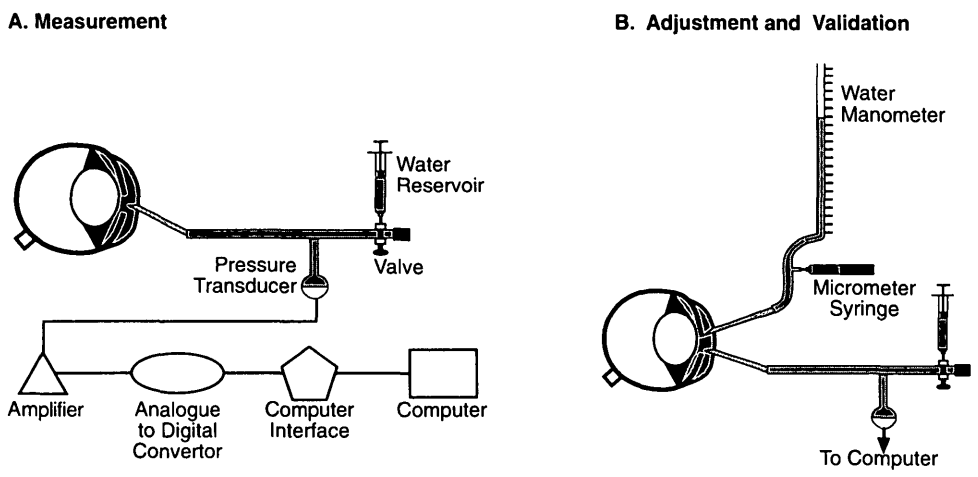

FIGURE 1. A diagrammatic representation of the equipment used to measure and adjust intraocular pressure.

chamber by piercing the cornea over the pupil. The microneedle was located precisely in the anterior chamber by use of a micromanipulator, on which it was mounted. The microneedle tip usually rested 50 μm to 100 μm into the chamber. Sometimes, however, the tip penetrated to approximately 250 μm to 300 μm and touched the lens. When this happened, the tip was retracted to a depth of 50 μm to 100 μm. Care was taken to minimize corneal deformation and to ensure that the eye remained in its normal position. The IOP was recorded at 30-second intervals for the first 2 minutes after ocular entry. These readings were averaged to determine the IOP of each animal. To confirm microneedle patency, gentle pressure was applied to the eyelid. A prompt increase in pressure was required for the inclusion of data. Carefully, the microneedle was withdrawn from the anterior chamber so that the tip was located in the drop of PBS. Rapid return of the pressure to zero was required for the inclusion of data. Before administering anesthesia to the next animal, the microneedle was flushed with sterile water. Procedures were similar in experiments involving the insertion of two microneedles. If penetration of the cornea was difficult and involved prolonged pushing (difficult entries), the data were discarded because the IOP may have been artificially low. Inclusion of data also required that an eye have minimal or no leakage. An eye was regarded as leaky if IOP readings dropped rapidly or consistently over the measurement period.

Slit Lamp Biomicroscopy. Pupils were dilated with a drop of 1% atropine sulfate, and eyes were viewed through a Nikon (Tokyo, Japan) biomicroscope. Eyes were analyzed at 1, 2, 6, and 8 weeks after IOP was measured.

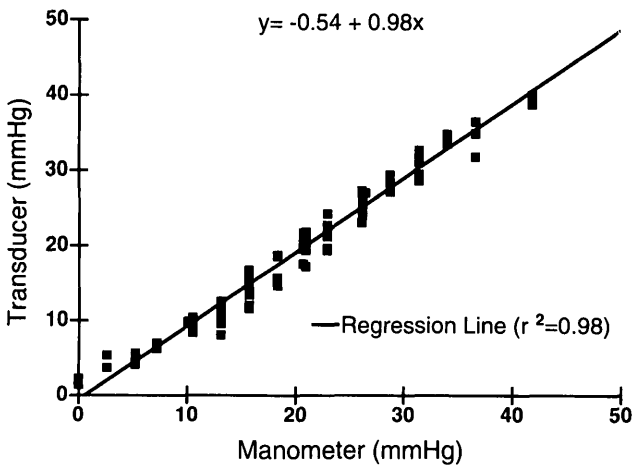

FIGURE 2. Scatterplot of manometrically set intraocular pressure (IOP) against transducer-generated IOP readings. The relationship between manometrically set IOP and transducer-measured IOP readings is shown.

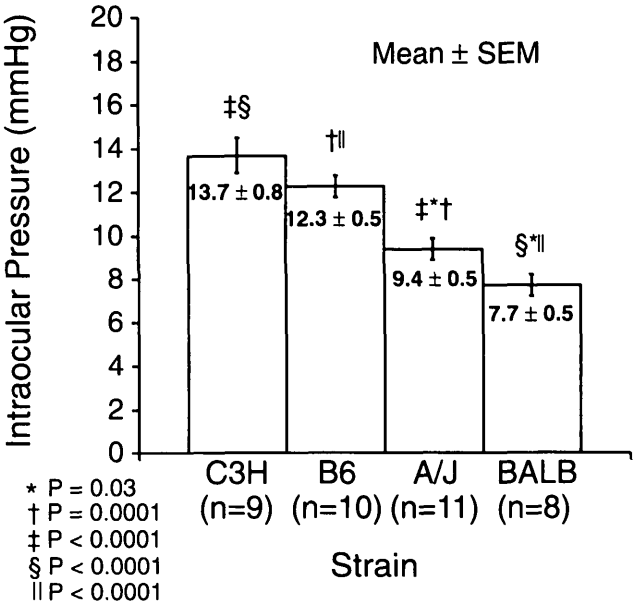

FIGURE 3. Intraocular pressure in male mice 9 to 11 weeks of age. Strain means were compared by a Student's *t*-test. The difference between strains C3H and B6 was not significant ($P > 0.1$). Other strain comparisons were as follows: *A/J versus BALB, ††A/J versus B6, ‡A/J versus C3H, §BALB versus C3H, and ‖BALB versus B6.

RESULTS. Validation and Success Rate. The reported system accurately measured the pressure (transducer pressure) in mouse eyes set at various pressures by use of a micrometer syringe and a water manometer (Fig. 2). Duplicate measurement systems were used to assess the effect of microneedle insertion. The pressure recorded on the first microneedle (IOP1), before insertion of the second microneedle, was compared to the pressure recorded through the second microneedle (IOP2). The process of inserting a microneedle into the anterior chamber did not significantly alter IOP. The mean difference between IOP1 and IOP2 was −0.3 ± 0.2 mm Hg ($P > 0.1$ for comparison of IOP1 and IOP2 by paired *t*-test). Corneal penetrations usually were uncomplicated; there was minimal leakage around the needle and often no detectable leakage at all. The average change in IOP for 15 eyes was −0.5 ± 0.1 mm Hg (median, −0.45 mm Hg) over the measurement period. We successfully measured IOP in 79% of the mice.

Intraocular Pressure. Figure 3 shows the intraocular pressure readings (mean ± SEM) of male mice of each strain. Strain means were compared by Student's *t*-test, and significant interstrain differences were found. Strains C3H and B6 have the highest pressures (13.7 ± 0.8 mm Hg and 12.3 ± 0.5 mm Hg, respectively). A/J is intermediate (9.4 ± 0.5 mm Hg), and BALB is the lowest (7.7 ± 0.5 mm Hg). All means except for C3H and B6 were significantly different.

Ocular Consequences. Very fine microneedles

damage the eye minimally. On needle removal, many eyes leaked but recovered quickly. Animals were monitored closely during the recovery period, and none showed any symptoms of distress (such as scratching or blepharospasm). Gross examination of the eyes revealed no damage 1 day after IOP measurement. Slit lamp examination identified a small scar (often not visible until pupil dilation) where the microneedle penetrated the cornea. In some animals, the microneedle touched the lens as it entered the eye, occasionally causing a small opaque spot on the lens. Everything else appeared normal by gross and slit lamp examination at 1, 2, 6, and 8 weeks after measurement.

DISCUSSION. We have developed and validated an accurate and reliable method to measure IOP in mice. To assess our system's ability to measure unknown IOP, we randomly varied IOP between 0 and 45 mm Hg and obtained transducer-generated readings in a "blinded" fashion. The close relationship between manometer and transducer readings (r^2 = 0.98) shows that the system accurately measures murine IOP over the tested range (Fig. 2). The effect of introducing the microneedles into the anterior chambers was evaluated by determining IOP with one microneedle and then observing the effect of inserting a second microneedle. Results show that microneedle insertion has only a minor effect on IOP (lowering it by 0.3 mm Hg on average) over the pressure range tested. The reliability of our procedure is demonstrated by the successful ascertainment of IOP in 79% of the experimental animals.

Using this system, we identified IOP differences between different inbred strains of mice (Fig. 3). Because these age- and sex-matched mice were maintained in virtually identical environments and were fed the same diet, these IOP differences probably were caused by interstrain genetic differences. The ability to measure genetically determined differences in IOP provides further evidence that this procedure is accurate and performs well in an experimental setting. In addition, it indicates that this method can be used in studies to map genes that contribute to IOP differences between various strains of mice.

The microneedle causes little visible damage to the analyzed eye, and animals exhibit no signs of pain or distress from it. Nevertheless, there are limitations to this invasive procedure for IOP evaluation. For example, the required use of anesthesia could alter IOP. For our analyses, we used a combination of ketamine and xylazine. Many anesthetic agents, including xylazine, lower IOP. Ketamine usually appears to increase IOP,[6-8] but there are reports of ketamine having no effect on IOP or even reducing IOP.[see 6,9] Different doses or different routes of administration may contribute to these differences. Few studies have assessed the physiological consequences of a mixture of ketamine and xylazine during the first 20 minutes after the administration of anesthesia. (In our experiments, IOP measurement usually was completed by 11 minutes after the administration of anesthesia.) Similarly, few studies have assessed these consequences after intraperitoneal administration (the preferred route of administration in mice), which usually takes longer to affect an animal's physiological status than direct intravenous or intramuscular injection. In one study, the effects of intravenous administration of ketamine and xylazine were evaluated in horses at 2-minute intervals until the animals started to wake (approximately 16 to 18 minutes after injection). Anesthesia had no significant effect on IOP.[10] This experiment was similar to ours because the mice woke from their dose of ketamine and xylazine between 15 and 20 minutes after injection. In another study, the effects of intraperitoneally administered ketamine and xylazine were evaluated in rats (we used the intraperitoneal route in mice). Anesthesia had a minor effect on blood pressure during the first 15 minutes after injection, but it had a strong hypotensive effect between 15 and 30 minutes that continued for more than 1 hour.[11] These experiments suggest that a combination of ketamine and xylazine causes relatively minor physiologic changes during the 15-minute interval immediately after the intraperitoneal administration of anesthesia (the time frame of all our IOP measurements). Supporting this is our unpublished observation that there was no significant difference in IOP between mice evaluated between 5 and 18 minutes after injection of our standard dose of these agents. Another drawback of our invasive method to measure IOP is the difficulty in interpreting repeated measurements in an eye after drug therapy because the microneedle may cause some inflammation and because aqueous humor often leaks from the eye on microneedle removal. These factors could alter aqueous humor dynamics for an unknown period of time. This is not a serious problem for genetic studies. Valuable data can be obtained from a single measurement in a number of genetically similar animals. In age-matched groups of animals that differ only in the presence or absence of a single mutation, IOP assessment can define the effects the mutation has on IOP. To determine the effects of the mutation on IOP with relation to age, different similarly matched groups of animals can be evaluated at different ages. A noninvasive method would be advantageous, however, and would allow repeated measurements on a single eye. A noninvasive measurement system would be more useful for investigating the effect of age, drugs, or other factors on IOP with time. Human tonometers have been used successfully to measure IOP in rats.[12,13] We have attempted to use both the Tono-Pen XL (Mentor, Santa

Barbara, CA) and the ProTon Tonometer (Tomey Technology, Cambridge, MA) on mice. In our hands, these instruments could not reproducibly measure IOP in the very small eyes of mice. We are working toward the development of a noninvasive tonometer for mouse eyes. Our current procedure will allow calibration and validation of future noninvasive instruments.

In summary, we have developed and validated a system that accurately measures IOP in mouse eyes. We have used this system to identify IOP differences between genetically distinct strains of mice. This work will facilitate studies on IOP and glaucoma in mice. These future studies will complement studies in humans and other species and will add considerably to a genetic understanding of glaucoma.

Key Words

genetics, glaucoma, intraocular pressure (IOP), mice

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Topical Corticosteroids Reverse the Antiviral Effect of Topical Cidofovir in the Ad5-Inoculated New Zealand Rabbit Ocular Model

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Purpose. To determine how the addition of topical corticosteroids would affect the anti-adenoviral inhibitory effect of topical cidofovir (S-HPMPC) in the Ad5 New Zealand (Ad5/NZ) rabbit ocular model.

Methods. In a series of experiments (two-eye design), Ad5-inoculated/NZ rabbits (10^6 pfu/eye) were treated with 1 of 3 treatment regimens. Group 1 was administered 1% cidofovir (CDV) twice a day for 3 days plus comfort tears four times a day for 14 days. Group 2 was administered 1% CDV twice a day for 3 days plus 1% Pred Forte four times a day for 14 days. Group 3 was administered vehicle twice a day for 3 days plus comfort tears four times a day for 14 days and served as the control. All eyes were evaluated for 21 days for serial eye titers, Ad5 positive eyes, and duration of Ad5 shedding.

Results. Compared to control eyes in the Ad5/NZ rabbit ocular model, CDV alone demonstrated a significant antiviral inhibitory effect: reduced mean Ad5 eye titer during the early phase of infection (days 3 to 7), fewer Ad5-positive eyes during the early and late (days 9 to 21) phases of infection, and shortened duration of shedding. However, concomitant treatment with both Pred Forte and CDV significantly reversed the antiviral